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Molecular support for the recognition of the *Mycoblastus fucatus* group as the new genus *Violella* (*Tephromelataceae*, *Lecanorales*)

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Abstract: The crustose lichen genus *Mycoblastus* in the Northern Hemisphere includes eight recognized species sharing large, simple ascospores produced 1–2 per ascus in strongly pigmented biatorine apothecia. The monophyly of *Mycoblastus* and the relationship of its various species to *Tephromelataceae* have never been studied in detail. Data from ITS rDNA and the genes coding for translation elongation factor 1-α and DNA replication licensing factor mini-chromosome maintenance complex 7 support the distinctness of *Mycoblastus* s. str. from the core of the *Tephromelataceae*, but recover *M. fucatus* and an undescribed Asian species as strongly supported within the latter group. We propose accommodating these two species in a new genus, *Violella*, which is characterized by its brownish inner ascospore walls, Fucatus-violet hymenial pigment granules and secondary chemistry, and discuss the position of *Violella* relative to *Calvitimela* and *Tephromela*. We describe the new species *Violella wangii* T. Sprib. & Goffinet to accommodate a new species with roccellic acid from Bhutan, China, India and the Russian Far East. We also exclude *Mycoblastus indicus* Awasthi & Agarwal from the genus *Mycoblastus* and propose for it the new combination *Malmidea indica* (Awasthi & Agarwal) Hafellner & T. Sprib.

Key words: ascus types, Asia, *Calvitimela*, EF1-α gene, fatty acid, lichens, *Malmidea*, Mcm7 gene, phylogeny, pigment, taxonomy

Introduction

The genus *Mycoblastus* is a widely distributed group of mainly epiphytic species found in cool temperate to arctic regions of both hemispheres. Its type species, *M. sanguinarius* (L.) Norman, is one of the common and familiar crustose lichens of boreal conifer forests, and is circumboreal. Despite being easily recognized and often collected, the genus has never been subjected to a complete global revision. Northern Hemisphere species concepts in *Mycoblastus* developed gradually through the description of forms and varieties of *M. sanguinarius* that were later raised to species rank. More species were added to the genus as regions of the Southern Hemisphere became better explored and species previously described under *Lecidea* were combined into *Mycoblastus* (e.g., Müller-Argoviensis 1894; Zahlbruckner 1926). Recent European taxonomic concepts and nomenclature were outlined by Schauer (1964), who recognized two species, and were expanded by James (1971), who provided a key. Recently Kantvilas (2009) revised cool temperate Southern Hemisphere material, recognizing eight species, which he considered to belong to two different species groups, the '*M. sanguinarius* group' which always contains atranorin, and the '*M. dissimulans* group', the members of which always contain perlatolic acid.

Mycoblastus in the Northern Hemisphere is currently considered to include eight species,

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namely *M. affinis*, *M. alpinus*, *M. glabrescens* (Kantvilas 2009), *M. sanguinarius*, *M. sanguinarioides* (Spribille *et al.* 2011), *M. japonicus* (Müller-Argoviensis 1891), *M. fucatus* (James 1971) and *M. caesius* (Tønsberg 1992). A dichotomy between atranorin- and perlatolic acid-containing species is present in the Northern Hemisphere as well, with *M. caesius* containing perlatolic acid and all other taxa containing atranorin and other substances. The atranorin-containing *Mycoblastus* species of the Northern Hemisphere have been accorded renewed attention recently with a detailed study of the *M. sanguinarius* group by Spribille *et al.* (2011). Specifically, these authors inferred the phylogenetic relationships with an emphasis on testing monophyly of *M. sanguinarius* in a phylogeny in which all known atranorin-containing Northern Hemisphere species were represented. *Mycoblastus fucatus* was represented by a single specimen, and was resolved to be only distantly related to the core group of *Mycoblastus*.

Mycoblastus fucatus is enigmatic among the Northern Hemisphere atranorin-containing species, for at least two reasons. First, its brilliant violet hymenial pigment, termed 'Fucatus-violet' by Kantvilas (2009), sets it apart from other *Mycoblastus* species, which contain the dull greenish to green-blue pigment 'Cinereorufa-green'. Second, it is the common and sole host of a lichenicolous fungus, *Tremella lichenicola*, which does not invade any other *Mycoblastus* species (Coppins & James 1979; Diederich 1986, 1996). Apart from James (1971), little attention has been paid to the ascocarps of *M. fucatus*, in part because they are so rare; in Norway, apothecia were observed in only three of 103 specimens studied by Tønsberg (1992). Sterile forms were described in Britain as a separate species, *M. sterilis* (Coppins & James 1979) until it was later realized that they were sterile forms of *M. fucatus* (Tønsberg 1992).

The recovery of *Mycoblastus fucatus* outside of the core of *Mycoblastus* by Spribille *et al.* (2011) motivated us to expand our sampling in line with our previous phylogenetic work on *Tephromela* s. lat. (Muggia *et al.* 2008), a lineage which has repeatedly been found to be related to *Mycoblastus* (Miądlikowska *et al.* 2006; Arup *et al.* 2007; Ekman *et al.* 2008). We also wanted to explore the possible relationship of *M. fucatus* with the saxicolous genus *Calvitimela* and some of the species groups discussed by Kantvilas (2009). Sequence motifs in *M. fucatus* indeed suggested affinities to *Tephromela* or *Calvitimela* rather than to *Mycoblastus*. At the same time, another taxon clearly related to *M. fucatus* was collected by the first two authors of this paper in Russia and China, providing more fresh material and further solidifying the concept of this as a recognizable species group with distinct morphological characters. Here, we present the results of molecular phylogenetic and morphological investigations on the *M. fucatus* group and propose for it the new genus *Violella*.

Materials and Methods

Taxon sampling and hypothesis testing

We designed our taxon sampling to include the core groups of *Mycoblastus* for which we could obtain fresh material, as well as representatives of major groups in the *Tephromelataceae* identified by Hertel & Rambold (1985), including *Tephromela*, *Calvitimela* and the "*Lecidea*" *aglaea* group, which has been treated as belonging to both *Tephromela* and *Calvitimela* in the past. We also generated sequences for several taxa of *Parmeliaceae*, which is a group often retrieved in BLAST searches of *Mycoblastus* sequences in GenBank. We included one specimen of *Japewia* (*Lecanoraceae*), hypothesized as being close to *Mycoblastus* by Kantvilas (2009), and spent some sequencing effort examining the possibility of relationships to *Megalaria*, also proposed as a relative of *Mycoblastus* by Kantvilas (2009), and *Psorinia*, suggested as a possible relative to *Calvitimela* by Hafellner & Türk (2001). We ultimately excluded *Megalaria* and *Psorinia* from our sampling because 1) morphological evidence, especially the strongly gelatinized proper exciple of *Megalaria*, argues against close relationships with that genus, and 2) DNA sequence data we obtained for single loci for both *Megalaria* and *Psorinia* were so different from the other taxa in our dataset as to be easily ruled out as close relatives. *Heppsora indica*, a species and genus described from Tamil Nadu state, India (Awasthi & Singh 1977; Singh & Sinha 2010: photograph), exhibits clear morphological affinities to *Tephromelataceae* (Poelt & Grube 1993). Unfortunately we did not have access to any fresh material; a specimen distributed under this name in a recent exsiccate

(Lumbsch & Feige, *Lecanoroid Lichens* #85) differs in chemistry and ascocarp pigmentation and is not *H. indica*. In the end, our taxon sampling (Table 1) provided a sufficient taxonomic neighbourhood to test the hypothesis of whether *Mycoblastus fucatus* would be recovered within *Mycoblastus*, in the vicinity of *Tephromela*, or in the vicinity of *Parmeliaceae* or *Lecanoraceae*.

Laboratory methods

Material for DNA extraction was taken from apothecia if present, otherwise from parasite-free thallus fragments inspected in water droplets on a microscope slide under ×20 magnification. Prepared material was transferred into reaction tubes, dried and pulverized using a TissueLyserII (Retsch). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) extraction kit using the manufacturer's instructions. For *Tephromela* specimens already studied by Muggia *et al.* (2008), existing extractions were used. Dilutions (mostly 5×10^{-2}) of the genomic DNA extractions were used as a template for the PCR reactions. After screening potential markers (Spribille *et al.* 2011), we settled on using three loci: two protein-coding genes, namely translation elongation factor 1-α (EF1-α) and the DNA replication licensing factor mini-chromosome maintenance complex 7 (Mcm7), and the nuclear ribosomal internal transcribed spacer region (ITS). For amplification of EF1-α from *Mycoblastus japonicus*, we employed a *Mycoblastus*-specific primer pair which will be described in detail elsewhere. PCR reactions were performed with Illustra Ready-To-Go RT-PCR Beads (GE Healthcare) in a thermocycler (AlphaMetrix) using conditions detailed by Spribille *et al.* (2011). Two µl aliquots of PCR products were viewed on 1% agarose gels stained with GelRed™ (Biotium, VWR); whole products were subsequently purified with NucleoSpin Extract II Kit (Macherey-Nagel). PCR product sequencing was outsourced to Macrogen, Inc. (Seoul, South Korea). Sequence fragments were obtained electronically from Macrogen and electropherogram ambiguities checked in BioEdit (Hall 1999). All DNA sequences were submitted to GenBank and are retrievable under the accession numbers listed in Table 1.

Phylogenetic analyses

Alignments were performed using ClustalW (Thompson *et al.* 1994) and subsequently optimized by hand in BioEdit (Hall 1999). Non-conserved regions and positions with missing data in >50% of sequences were removed using Gblocks (Talavera & Castresana 2007). Candidate nucleotide substitution models were identified for each partition using the likelihood ratio test implemented in jModelTest (Posada 2008); likelihood scores were then compared based on the Akaike Information Criterion (AIC). Individual gene alignments were analyzed using a maximum likelihood (ML) and Bayesian Markov Chain Monte Carlo (B/MCMC) approach. We tested for conflict between partitions by examining frequencies of bipartitions for the same taxon sets across all three partitions using a set of B/MCMC gene trees; a conflict was interpreted as significant if two well supported different relationships were detected for the same taxon set (Kauff & Lutzoni 2002); we used the threshold of $\geq$ 95%. Maximum likelihood analyses were performed using the program PhyML 3.0 (Guindon *et al.* 2010). Bootstrapping was carried out on 500 tree replicates. B/MCMC analyses were performed using the program MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) using substitution models approximated by jModeltest (see above). For each analysis, two runs with ten million generations each starting with a random tree and running four simultaneous chains was employed. Every 1000th tree was sampled and saved to a file. The first 5 000 000 generations (5000 sampled trees) were discarded as chain 'burn-in'. Of the remaining 5001 trees a majority consensus tree with averaged branch lengths and annotated with posterior probability values at every node was calculated using the sumt command in MrBayes. The program TRACER v. 1.5 (http://tree.bio.ed.ac.uk/software/tracer/) was used to assess whether likelihood values had reached stationarity within the allocated burn-in window by plotting log likelihood against the number of generations. In addition, we examined the distributions of split frequencies using the online program AWTY (Nylander *et al.* 2007) to test whether runs had converged. Only clades that received bootstrap values $\geq$ 70% in ML and posterior probabilities $\geq$ 0·95 were considered significantly robust. Phylogenetic trees were visualized in TreeView (Page 1996).

Morphological and chemical analyses

To test whether our phylogenetic results could be matched by morphological traits, we sorted specimens under a Leica Wild M3Z dissecting microscope and examined anatomical sections on material mounted in water with a Zeiss Axioskop light microscope fitted with Nomarski differential interference contrast and outfitted with a ZeissAxioCam MRc5 digital camera. Some images were digitally optimized through 'stacking' using CombineZM open source image processing software (www.hadleyweb.pwp.blueyonder.co.uk/CZM/). Ascospore, areole, soredia and apothecia measurements are given as (smallest absolute measurement–)smallest average – largest average(–largest absolute measurement). Ascus morphology was investigated in asci with immature ascospores (following Hafellner 1984). In addition, we examined specimens for chemical patterns that could corroborate phylogenetic differentiation using thin-layer chromatography (TLC), following the methods of Culberson (1972) with modifications (Culberson & Johnson 1982). We used silica-coated glass plates (Macherey-Nagel 821 030) run their full length in solvent systems A, B' and C. Aliphatic acids were visualized by immersing completely dried plates post-development into a tank of water for 1–2 s, quickly dripping off the plates and marking spots over the next 4 min. No attempt was made to separate roccellic and angardianic acids, which are indistinguishable in TLC (Tønsberg 1992).

TABLE 1. *DNA vouchers and GenBank Accession Numbers of the species used in this study; bold species names and accession numbers indicate new accessions*

Species	Ref. number	Voucher	GenBank Accession Numbers		
			EF1-α	ITS	Mcm7
Alectoria sarmentosa	**638**	**Canada**, British Columbia, near mouth of Halfway River on Upper Arrow Lake, 2009, *Spribille* s.n. (GZU)	**JN009675**	**JN009706**	**JN009737**
Allantoparmelia sibirica*	**854**	**USA**, Alaska, Dalton Highway, Finger Mtn., 2010, *Spribille* s.n. (GZU)	**JN009676**	**JN009707**	–
Calvitimela armeniaca	**599**	**Canada**, Yukon, Mt. Martin, *Spribille* 28707 (GZU)	**JN009677**	**JN009708**	**JN009738**
C. armeniaca	**607**	**Austria**, Carinthia, Koralpe, *Hafellner* 71304 (GZU)	**JN009678**	**JN009709**	**JN009739**
C. armeniaca	**836**	**Spain**, Catalonia, Parque Nacional de Aigüestortes i Estany De Sant Maurici, *Pérez-Ortega* 1321 (GZU)	–	**JN009710**	**JN009740**
C. armeniaca	**837**	**Spain**, Catalonia, Parque Nacional de Aigüestortes i Estany De Sant Maurici, *Pérez-Ortega* 1322 (GZU)	–	**JN009711**	**JN009741**
C. armeniaca	**856**	**USA**, Alaska, Dalton Highway, Finger Mtn., 2010, *Spribille* s.n. (GZU)	**JN009679**	**JN009712**	**JN009742**
C. melaleuca	**150**	**USA**, Alaska, White Pass, *Spribille* 26952 (KLGO)	**JN009680**	**JN009713**	**JN009743**
C. melaleuca	**838**	**USA**, Alaska, Alaska Range, Mt. Healy, *Spribille* 27965-B (GZU)	–	**JN009714**	**JN009744**
Cetraria sepincola	**639**	**Slovakia**, Nizke Tatry, between Čertovica and D'umbieř, *Spribille* 32131 *&* *Wagner* (GZU)	**JN009681**	**JN009715**	**JN009745**
Japewia subaurifera	**764**	**USA**, New Hampshire, Coos Co., ridge S of Dixville Notch, 2009, *Spribille & Wagner* s.n. (GZU)	**JN009682**	**JN009716**	–
"Lecidea" aglaea	**608**	**Austria**, Vorarlberg, Rätikon, *Hafellner* 72944 (GZU)	**JN009683**	**JN009717**	–
"Lecidea" aglaea	**847**	**Austria**, Styria, Koralpe, *Hafellner* 70358 (GZU)	**JN009684**	**JN009718**	–
"Lecidea" aglaea	**867**	**Sweden**, Jämtland, Åre par., Mt. Skurdalsbergen, *Nordin* 6659 (UPS-183008)	**JN009685**	**JN009719**	–
Miriquidica instrata	**852**	**USA**, Montana, Lincoln Co., Whitefish Range, Lewis Creek talus, 2010, *Spribille* s.n. (GZU)	**JN009686**	**JN009720**	**JN009746**
Mycoblastus affinis	90	**Canada**, British Columbia, Philipp Lake, 2008, *Goward & Wright* s.n. (GZU)	JF744895	JF744969	JF744809
M. affinis	121	**USA**, Alaska, Russian River, *Spribille* 27371 (GZU)	JF744896	–	JF744812
M. affinis	379	**USA**, Montana, Lincoln Co., Laughing Water Creek, *Spribille* 30126 (GZU)	JF744898	JF744980	JF744795
M. affinis	420	**Austria**, Styria, near Oberzeiring, *Spribille* 30220 (GZU)	JF744899	JF744978	JF744797
M. affinis	464	**Germany**, Bavaria, Bayrischer Wald, Dreisesselfels, *Spribille* 32115 *&* *Wagner* (GZU)	JF744900	JF744979	JF744800

TABLE 1. *Continued*

Species	Ref. number	Voucher	GenBank Accession Numbers		
			EF1-α	ITS	Mcm7
M. affinis	465	**Austria**, Styria, Hörsterkogel, *Spribille* 32102 (GZU)	JF744902	JF744977	JF744801
M. affinis	766	**Canada**, Nova Scotia, Cape Breton, 2009, *Spribille & Wagner* s.n. (GZU)	JF744897	–	JF744813
M. affinis	**795**	**China**, Yunnan, *Goffinet* 10030 (CONN)	–	**JN009721**	**JN009747**
M. affinis	**858**	**Canada**, Québec, Gaspesie E of Claridorme, 2009, *Spribille & Wagner* s.n. (GZU)	–	**JN009722**	–
M. alpinus†	466	**Canada**, Yukon, LaBiche River area, *Spribille* 28541 (GZU)	JF744903	–	JF744802
M. alpinus	537	**Canada**, Québec, Lac à Jack, 2009, *Spribille & Clayden* s.n. (GZU)	JF744901	JF744976	JF744805
M. alpinus	468	**USA**, Alaska, White Pass, *Spribille* 26781 (KLGO)	JF744904	–	JF744803
M. glabrescens	92	**USA**, Washington, Skamania Co., Elk Pass, *Spribille* 29848 (GZU)	JF744894	JF744967	JF744810
M. glabrescens	352	**USA**, Idaho, Shoshone Co., Hobo Cedars, *Spribille* 30024 (GZU)	JF744893	JF744985	JF744816
M. glabrescens	367	**USA**, Oregon, Linn Co., Tombstone Pass, *Spribille* 29899 (GZU)	JF744892	JF744984	JF744815
M. japonicus	**802**	**South Korea**, Gangwon Prov., Sorak-san National Park, *Thor* 20551 (UPS)	**JN009688**	JF744983	–
M. sanguinarioides	250	**USA**, Alaska, Chilkoot Trail, *Spribille* 27038-A (GZU)	JF744884	JF744971	JF744794
M. sanguinarioides	460	**Russia**, Khabarovskiy Krai, 10 km W of De Kastri, *Spribille* 30655 (GZU)	JF744886	JF744974	JF744799
M. sanguinarioides‡	**502**	**Japan**, Hokkaido, Prov. Kushiro, Mt. O-akan, *Ohmura* 6740 (GZU)	**JN009689**	**JN009723**	**JN009748**
M. sanguinarioides	542	**Canada**, Nova Scotia, Advocate Harbour, 2009, *Spribille & Wagner* s.n. (GZU)	JF744888	JF744981	JF744806
M. sanguinarioides	582	**Australia**, Tasmania, foot of Adams Peak, *Kantvilas* 1/09 (GZU)	JF744889	JF744972	JF744819
M. sanguinarioides‡	**857**	**Japan**, Honshu, Mt. Fuji, *Ohmura* 5996 (GZU)	**JN009690**	**JN009724**	–
M. sanguinarius	100	**Canada**, British Columbia, Retallack, *Spribille* 30134-A *& Pettitt* (GZU)	JF744879	JF744913	JF744746
M. sanguinarius	120	**USA**, Alaska, Russian River, *Spribille* 27370 (GZU)	JF744827	JF744914	JF744747
M. sanguinarius	170	**Norway**, Hordaland, Åsane, *Spribille* 30237-I (GZU)	JF744843	JF744905	JF744765

TABLE 1. *Continued*

Species	Ref. number	Voucher	GenBank Accession Numbers		
			EF1-α	ITS	Mcm7
M. sanguinarius	236	**USA**, Oregon, Wasco Co., along Hwy. 26, *Spribille* 29881-C (GZU)	JF744858	JF744944	JF744777
M. sanguinarius	410	**Russia**, Khabarovskiy Krai, Etkil'-Yankanskiy Mountains, *Spribille* 31330 (GZU)	JF744864	JF744949	JF744781
M. sanguinarius	**436**	**Russia**, Khabarovskiy Krai, near Lazarev, *Spribille* 30949 (GZU)	**JN009691**	JF744950	JF744782
M. sanguinarius	**486**	**Sweden**, Pite Lappmark: Arvidsjaur par., 13 km NNW of Moskosel, *Muggia* (TSB-38893)	**JN009692**	**JN009725**	–
M. sanguinarius	493	**Japan**, Hokkaido, Prov. Kushiro, Mt. O-akan, *Ohmura* 6746 (GZU)	JF744866	JF744953	JF744786
M. sanguinarius	543	**Canada**, Québec, Rte. 138 N of Les Escoumins, 2009, *Spribille & Clayden* s.n. (GZU)	JF744856	JF744956	JF744787
M. sanguinarius	**590**	**Russia**, Chelyabinskaya Oblast', Zyuratkul' National Park, Khrebet Nurgushch, 31 May 2009, *Urbanavichene* s.n. (GZU)	**JN009693**	–	**JN009749**
M. sanguinarius	598	**Russia**, Leningrad Oblast', 7·5 km E of Ladva Village, 2009, *Stepanchikova* s.n. (GZU)	JF744869	JF744961	JF744792
M. sanguinarius	**605**	**Canada**, Yukon, LaBiche River area, *Spribille* 28305 (GZU)	JF744877	JF744987	**JN009750**
M. sanguinarius	GB1	**Canada**, Québec, Rivière Noire, *Lutzoni & Miądlikowska* (DUKE-47513)	DQ782898	DQ782842	–
M. sanguinarius	**MS15**	**Russia**, Primorskiy Krai, Oblachnaya, *Spribille* 23583 *& Krestov* (BG)	**JN009694**	**JN009726**	–
M. sanguinarius	**772**	**Russia**, Khabarovskiy Krai, Bureinskiy Zapovednik, near Staraya Medvezhka, *Spribille* 31959 *& Yakovchenko* (GZU)	**JN009695**	**JN009727**	**JN009751**
Protoparmelia badia	**853**	**USA**, Montana, Lincoln Co., Whitefish Range, Lewis Creek talus, 2010, *Spribille* s.n. (GZU)	**JN009696**	**JN009728**	**JN009752**
Tephromela atra	**L415**	**Greece**, Crete, Herakleion, Kameraki, *Muggia* (TSB 37924)	**JN009697**	EU558688	**JN009753**
T. atra	**L223**	**Italy**, Campania, Napoli, Capri Island, *Muggia* (TSB 37119)	**JN009698**	EU558648	**JN009754**

TABLE 1. *Continued*

Species	Ref. number	Voucher	GenBank Accession Numbers		
			EF1-α	ITS	Mcm7
T. atra	**L228**	**Italy**, Campania, Napoli, Capri Island, *Muggia* (TSB 37124)	**JN009699**	EU558650	**JN009755**
T. atra	**L248**	**Italy**, Campania, Napoli, Capri Island, *Muggia* (TSB 37133)	–	EU558656	**JN009756**
T. atra	**L284**	**Italy**, Sardinia, Nuoro, Mt. Albo, *Muggia (*TSB 37465)	–	EU558661	**JN009757**
T. atra calcarea	**628**	**Greece**, Epirus, Tzoumerka, *Spribille* 15951 (GZU)	**JN009700**	**JN009729**	**JN009758**
T. atra calcarea	**L403**	**Greece**, Crete, Lasithi, Selakano forest, *Muggia* (TSB 37912)	–	EU558681	**JN009759**
T. atra calcarea	**L280**	**Italy**, Sardinia, Nuoro, Mt. Albo, *Muggia* (TSB 37461)	–	EU558660	**JN009760**
T.* cf. *pertusarioides[§]	**850**	**Russia**, Khabarovskiy Krai, Bureinskiy Zapovednik, near Staraya Medvezhka, *Spribille* 31797 *& Yakovchenko* (GZU)	**JN009701**	**JN009730**	**JN009761**
Tephromela sp. Björk 18057[¶]	629	**Canada**, British Columbia, Fraser Canyon, *Björk* 18057 (UBC)	JF744875	JF744986	JF744821
Usnea intermedia	**609**	**Austria**, Styria, Gurktaler Alpen, *Obermayer* 11839 (GZU)	**JN009702**	**JN009731**	**JN009762**
Violella fucata	**844**	**Germany**, Bavaria, Bayerischer Wald, Dreisesselfels, *Spribille* 32112 (GZU)	–	**JN009732**	–
V. fucata	**600**	**USA**, Massachusetts, Mt. Greylock, *Spribille* 32161 (GZU)	**JN009703**	JF744968	JF744818
V. fucata	**835**	**Slovenia**, Snežnik area, *Spribille* 30276 *& Mayrhofer* (GZU)	–	**JN009733**	**JN009763**
V. wangii	**796**	**China**, Yunnan, Laojunshan, *Goffinet* 10029 (KUN)	**JN009704**	**JN009734**	**JN009764**
V. wangii	**842**	**China**, Yunnan, Laojunshan, *Goffinet* 10033 (UPS)	–	**JN009735**	**JN009765**
V. wangii	**840**	**Russia**, Khabarovskiy Krai, Chegdomyn-Sofiysk road, *Spribille* 31621 *& Yakovchenko* (H)	**JN009705**	**JN009736**	**JN009766**

[⋆]first confirmed record for North America (TLC: α-collatolic and alectoronic acids)
[†]reported as *M. affinis* by Spribille *et al.* (2011), this specimen actually corresponds to the *alpinus* morphotype
[‡]first modern record for Japan
[§]first record for Russia
[¶]previously published as *T. atra* s.lat. by Spribille *et al.* (2011), but probably an undescribed taxon

Pigments were examined under the light microscope and named according to Meyer & Printzen (2000), except for Fucatus-violet, which was not treated by those authors. Fucatus-violet would key in Meyer & Printzen's key under lead 2 as N+ violaceous as it goes from its natural violet colour in H_2O to a deep raspberry red. It has the following standard reactions: K+ peacock-blue, N+ raspberry-red, HCl− (slowly fading but maintaining hue), C+ grey, eventually bleaching altogether; after pretreatment with N: K greenish yellow ↔ HCl completely clear. The pigment was mentioned already by Stirton (1879) as an 'intense violaceous colour' and has also been previously referred to as 'gentian violet' (James 1971; James & Watson 2009). We adopt the name proposed by Kantvilas (2009).

Reference material studied for morphological comparisons. Calvitimela armeniaca (DC.) Hafellner: **Austria:** *Carinthia*: Koralpe, *c.* 12 km NE above St. Paul in Lavanttal, 2008, *Hafellner* 71304 *&* *Hafellner* (GZU).

"*Lecidea*" *aglaea* Sommerf.: **Austria:** *Styria*: Koralpe, *c.* 15·5 km WNW of Deutschlandsberg, 2007, *Hafellner* 70358 (GZU); Vorarlberg, Rätikon, 2008, *Hafellner* 72944 (GZU).

Mycoblastus dissimulans (Nyl.) Zahlbr.: **Chile:** *Region de los Lagos*: Isla Grande de Chiloé, 2009, *Pérez-Ortega* 1186 *&* *Etayo* (GZU).

Mycoblastus sanguinarius (L.) Norm.: **USA:** *Alaska*: Kenai Peninsula, Russian River, 2008, *Spribille* 27359 *&* *Wright* (GZU).

Tephromela atra (Huds.) Hafellner: **Greece:** *Epirus*: Tzoumerka, near Kataraktis, Shrine of Profitis Ilias, 2005, *Spribille* 16260 (GZU).

Results of phylogenetic analysis

We obtained 91 new DNA sequences from 43 individuals, including 30 of EF1-α, 31 of ITS and 30 of Mcm7. Following exclusion of positions with missing or ambiguous data, the sequences consisted of 852, 478 and 564 characters, respectively, for a combined total of 1894 characters. Tests of nucleotide substitution models returned TIM3ef+I+G for EF1-α, GTR+I+G for ITS, and HKY+I+G for Mcm7. We ran individual B/MCMC analyses for each locus but detected no significant conflict between the loci, and thus combined them. Our partitioned B/MCMC analysis employed six, six and two substitution rate categories, respectively, for the three partitions; four rate categories, predicted in the TIM3ef model, are not possible to implement in current software. Overall rate heterogeneity was modelled using a gamma density function. ML and B/MCMC returned congruent phylogenies for the concatenated data set. Analysis of B/MCMC log likelihood outputs in Tracer indicated that convergence was reached well before our burn-in threshold; plotting of split frequencies between runs in AWTY also showed stationarity had been reached. The average standard deviation across runs for splits with a frequency of at least 0·1 was 0·003493.

We recovered two strongly supported core groups (Fig. 1), one of which includes *Tephromela*, *Calvitimela* s.lat. and the *Mycoblastus fucatus* group (which we call here 'core *Tephromelataceae*'), and another including *Mycoblastus* s.str. Both of these clades were separated from the five taxa of *Parmeliaceae* at the base of the tree and *Japewia subaurifera*, which was recovered close to *Miriquidica instrata* (*Lecanoraceae*). The combined *Mycoblastus* clade consists of a strongly supported monophyletic *M. sanguinarius*, *M. sanguinarioides* and *M. glabrescens*. *Mycoblastus alpinus* was recovered within a strongly supported *M. affinis* clade and the single individual of *M. japonicus*, which for the first time is represented by two markers in a molecular phylogeny, is recovered as strongly supported sister to *M. affinis*.

The 'core *Tephromelataceae*' clade consists of four distinct, well supported groups; the relationships to each other are, however, not supported. These groups correspond to *Tephromela* (*T. atra*, *T.* cf. *pertusarioides* and the undescribed *Tephromela* sp. Björk 18057), *Calvitimela* s.str. (*C. armeniaca* and *C. melaleuca*), the *Mycoblastus fucatus* group, interpreted here as the new genus *Violella* (see below), and "*Lecidea*" *aglaea* on its own long branch separate from the rest of *Calvitimela*.

Discussion

Hertel & Rambold (1985) provided an overview of species groups in what they considered *Tephromela*, and later Kantvilas (2009) proposed a range of potential relatives for *Mycoblastus*. Our results shed new light on potential relationships and invite a reassessment of meaningful morphological characters (Table 2). In his study of Lecanoralean

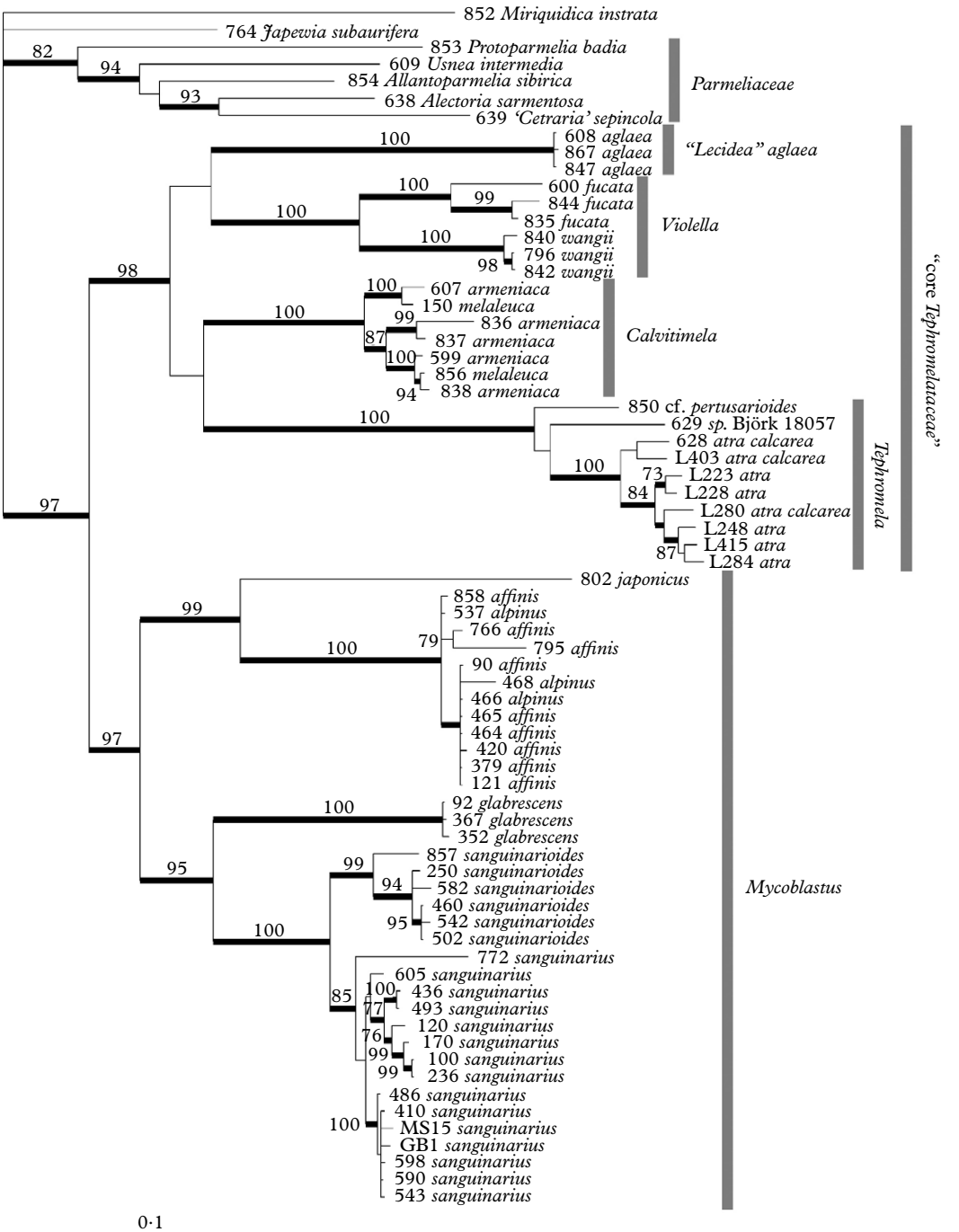

FIG. 1. Majority rule B/MCMC consensus tree of the concatenated EF1-α , ITS and Mcm7 data set. Posterior probabilities ≥95% are shown as thick branches; bootstrap support results of maximum likelihood analysis are shown where ≥70%. Reference numbers refer to Table 1.

ascus types, Hafellner (1984) implied deep differences between *Tephromela* and *Mycoblastus*, sufficient for him to recognize them as belonging to different families, *Tephromelataceae* and *Mycoblastaceae*. Indeed, our results strongly support the distinctness of *Mycoblastus s. str.* from a 'core *Tephromelataceae*' (Fig. 1). This does not necessarily translate to different families, however. We did not structure the taxon sampling of our phylogenetic analysis to test family-level relationships within a broader Lecanoralean context, and cannot predict the outcome of such a study. Morphologically, however, the distinction of two families would appear to be untenable. *Mycoblastus* shares a similar ascus apical apparatus with members of *Tephromelataceae*, similar development of a peculiar thalline cushion below the apothecia (see below), similar pycnidial development, conidiophores, shared ascocarp pigments and widely overlapping thallus secondary chemistry. Morphologically, the only difference we have found may relate to the basic type of hymenial matrix formed by the paraphyses. In 'core *Tephromelataceae*', paraphyses can be branched and anastomosing, but more often than not they form long, straight, multicellular 'beams' that separate easily in K and are substantially thicker than the cross-bridges (Fig. 2F). In *M. sanguinarius*, by contrast, paraphyses almost never form straight segments even within a single paraphysis cell, the anastomosing network is intricate, with bridges often nearly as thick as the main beams (Fig. 2E), and the entire network enmeshes the asci; even in K, squashing of the hymenium results in breakage of the hymenium rather than separation of asci and paraphyses. We never found the extreme degree of branching and anastomosing without straight beams depicted by James (1971: fig. 7) for *M. fucatus* but instead always found the paraphysis beams to be much thicker than the bridges and easily separable in K, and thus similar to other core *Tephromelataceae*.

Another enigmatic structure linking *Tephromelataceae* and *Mycoblastus* is the so-called thalline exciple, especially evident in *Tephromela*. Hertel & Rambold (1985) and Kantvilas (2009) have interpreted the 'thalline exciple' of *Tephromela* to be homologous, or at least worthy of providing in the same table category, to the proper exciple in other genera. We have, however, found apparently homologous thalline tissue, in addition to the presence of a rudimentary proper exciple, in all genera of *Tephromelataceae* and *Mycoblastus*. We hesitate to refer to this as an amphithecium or thalline exciple because it lacks an algal layer and consists of differentiated, dense, prosoplectenchymatous tissue not normally found in the thallus. Instead we will refer to it as a 'thalline cushion'. The thalline cushion occasionally emerges to outer view as a thin or thick white line in *M. sanguinarioides* (T. Spribille, unpublished data), is visible in section in the *M. fucatus* group (Fig. 3C & 3F), and in *Tephromela* it forms a 'thalline rim'. However, it is even present in *Calvitimela*, where it forms a dense layer between the subhymenium and the thallus medulla.

Our phylogenetic results re-open a discussion on the generic boundaries in *Tephromelataceae*, begun by Hertel & Rambold (1985) and continued by Hafellner & Türk (2001), with the description of *Calvitimela*. *Tephromela* possesses *Biatora*-type asci with a sometimes bulbous masse axiale (Fig. 2B). Hafellner & Türk (2001) separated out *Calvitimela* in part based on its *Lecanora*-type ascus, though even in describing their new genus they already anticipated that the "*Lecidea*" *aglaea* group, with its *Biatora*-type asci (Fig. 2C), might not be closely related to the type species *C. armeniaca* (Fig. 2A). Even so, they transferred it to *Calvitimela*. Our results confirm that the two are not closely related and we thus maintain this taxon in the genus *Lecidea* in the broad sense until its generic disposition can be resolved. To this medley can now be added the *M. fucatus* group with its *Biatora*-type asci (Fig. 2D). *Mycoblastus fucatus* has long been recognized for its unusual hymenium pigmentation, a character absent from *Mycoblastus* s. str. Furthermore, *M. fucatus*, and in particular material from Asia that will be described here as a new taxon, possesses a character not known from any of the other associated genera studied here, namely the tendency of the

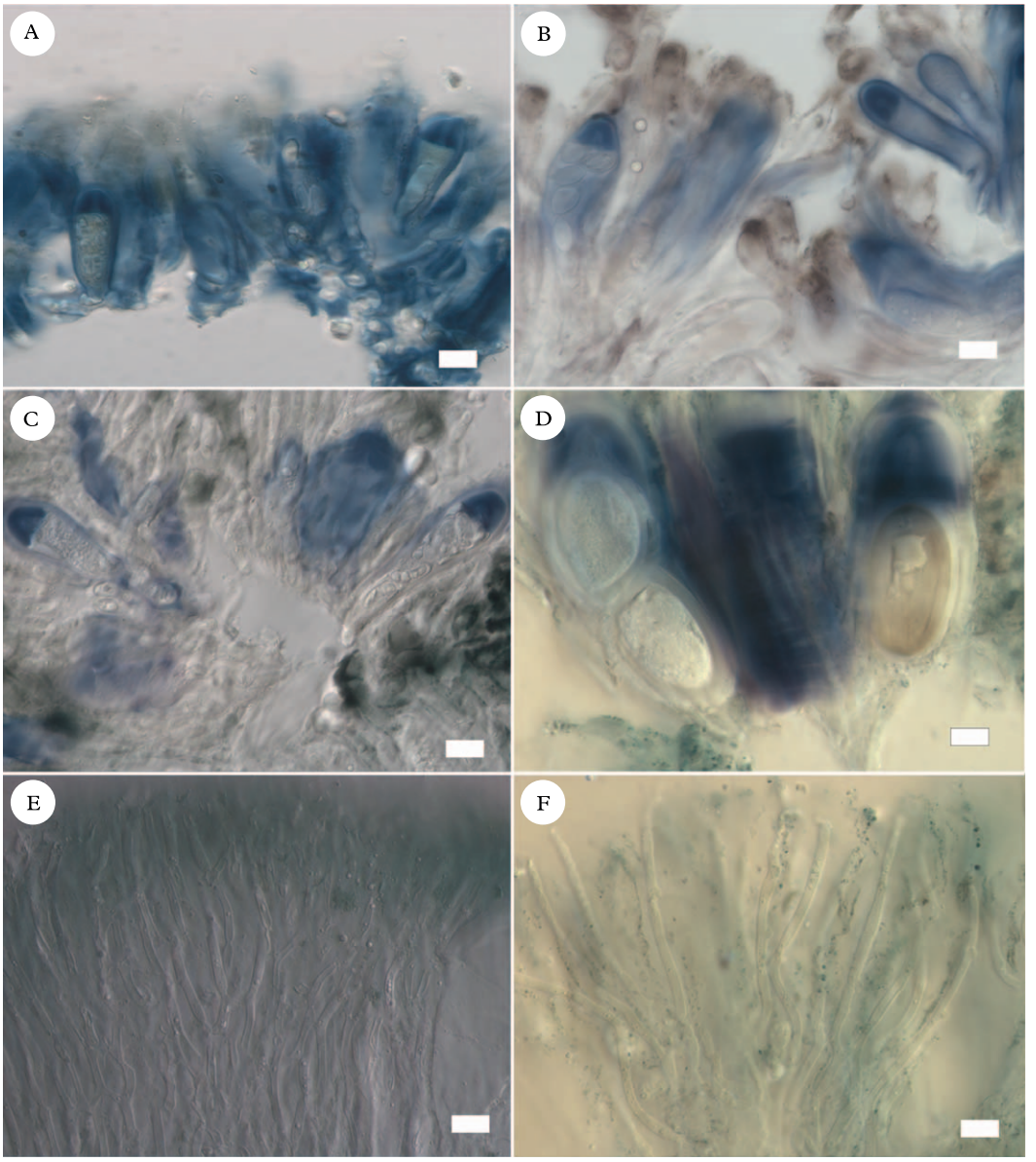

FIG. 2. Selected asci and paraphyses. A–D, ascus variation in the *Tephromelataceae*, showing asci with immature ascospores; A, *Calvitimela armeniaca* (*Hafellner* 71304); B, *Tephromela atra* (*Spribille* 16260); C, "*Lecidea*" *aglaea* (*Hafellner* 72944); D, *Violella wangii* (holotype). E & F, paraphyses; E, *Mycoblastus sanguinarius* (*Spribille* 27359); F, *Violella wangii* (holotype). A–D in $I_{Lugol's}$ after pretreatment with K, E & F in K. Scales: A–F = 10 µm.

internal ascospore wall to turn brown. This character was already noted by Leighton (1879, see also below). These characters also do not reconcile with those of *Tephromela* and *Calvitimela*, which differ in hymenium pigmentation, ascus type and, in part, secondary chemistry (Table 2). We accordingly propose recognizing *M. fucatus* and this new taxon as constituting the new genus *Violella*. The alternative generic solution would require all

TABLE 2. Characters of genera and major groups in the *Tephromelataceae* and *Mycoblastus*

	Violella	*Calvitimela*	*"Lecidea" aglaea* group	*Heppsora*⋆	*Tephromela*	*Mycoblastus*	*M. dissimulans*
Ascospore walls melanizing when old	yes, in endospore	no	no	no	no	no	no
Ascospore walls	double†	apparently single	apparently single	apparently single	apparently single	double	double
Ascus wall in $I_{Lugol's}$	moderately amyloid, internal content visible	weakly amyloid, internal content clearly visible	weakly amyloid, internal content clearly visible	not studied	weakly amyloid, internal content clearly visible	strongly amyloid, internal content concealed except when iodine dissipates	strongly amyloid, internal content concealed except when iodine dissipates
Ascus apical apparatus	*Biatora*-type	*Lecanora*-type	*Biatora*- to *Bacidia*-type	±*Lecanora*-type	±*Biatora*-type	*Biatora*- to *Bacidia*-type	±*Bacidia*-type
Ascus ocular chamber at median development	*c.* 1/4 to 1/5 of ascus length	*c.* 1/5 of ascus length	*c.* 1/5 of ascus length	not studied	*c.* 1/5 of ascus length	*c.* 1/3–1/4 of ascus length	*c.* 1/3–1/4 of ascus length, ascus often becoming pyriform
Number of ascospores per ascus	mostly 2 (1–3)	8	8	8	8	1–2	2
Paraphyses	stout with thin cross-bridges	stout with thin cross-bridges	stout with thin cross-bridges	not studied	stout with thin cross-bridges	netted, cross-bridges of similar thickness to main beams	netted, cross-bridges of similar thickness to main beams

TABLE 2. *Continued*

	Violella	*Calvitimela*	*"Lecidea" aglaea* group	*Heppsora*⋆	*Tephromela*	*Mycoblastus*	*M. dissimulans*
Hymenial pigmentation	Fucatus-violet, secondary Cinereorufa-green	Cinereorufa-green	Cinereorufa-green	Atra-red	Atra-red	Cinereorufa-green	Cinereorufa-green‡
Proper exciple	reduced, hyphae similar to paraphyses	reduced, hyphae similar to paraphyses	reduced, hyphae similar to paraphyses	reduced, hyphae similar to paraphyses	reduced, hyphae similar to paraphyses	reduced, hyphae similar to paraphyses	reduced, hyphae similar to paraphyses
'Thalline cushion'	rudimentary to well developed and forming ring around apothecia	rudimentary, thin layer below proper exciple	rudimentary, thin layer below proper exciple	highly reduced or appearing absent	well developed and forming 'thalline margin'§	rudimentary to well developed and forming ring around apothecia	rudimentary, thin layer below proper exciple
Conidia	bacilliform	bacilliform¶	ellipsoid to bacilliform¶	bacilliform	filiform¶	bacilliform	bacilliform
Thallus morphology	crustose	crustose	crustose	peltate-squamulose	crustose to fruticose**	crustose	crustose
Thallus secondary chemistry	atranorin, fumarprotocetraric acid + fatty acid	alectorialic acid, psoromic acid, stictic acid + fatty acids	atranorin, usnic acid + fatty acids	atranorin, alectronic and α-collatolic acid	atranorin, alectronic acid, α-collatolic acid, physodic acid and rarely fatty acids	atranorin, planaic, fumarprotocetraric + fatty acids	perlatolic acid + fatty acids

⋆description based on Awasthi & Singh (1977) and Poelt & Grube (1993);
†outer wall considered an epispore by Stirton (1879), but not dissolving in C;
‡Fucatus-violet not seen in Chilean material but reported from Tasmania by Kantvilas (2009);
§'exciple' of Kantvilas (2009, p. 158: table);
¶illustrated by Hertel & Rambold (1985);
**in *T. siphuloides* (Poelt & Grube 1993).

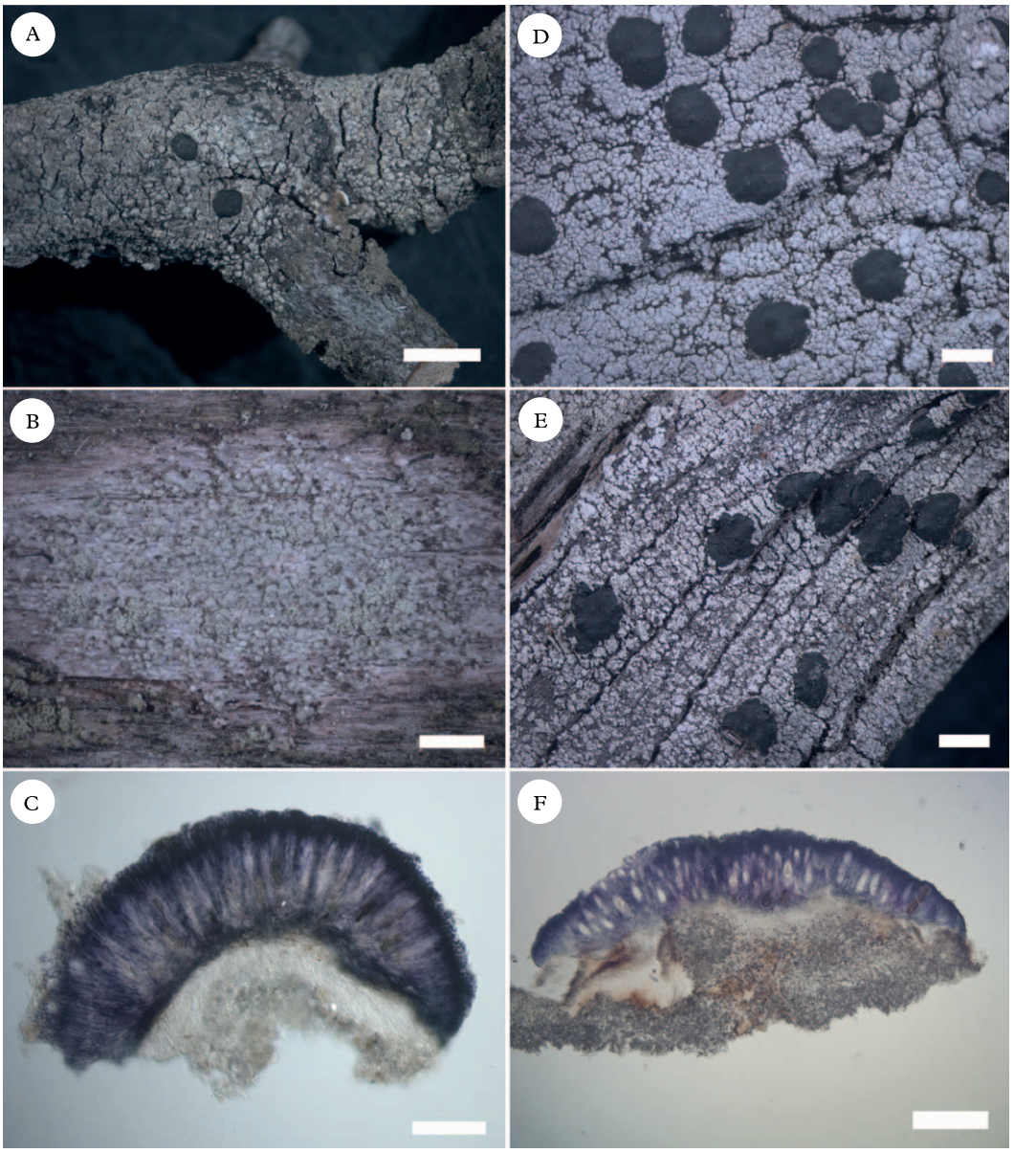

FIG. 3. *Violella* species, habit. A–C, *V. fucata*; A, fertile specimen (*Tønsberg* 19004); B, sterile specimen on wood (*Spribille* 32161); C, section of apothecium, in water (*Tønsberg* 19004). D–F, *V. wangii*; D, (holotype); E, sorediate morph (*Spribille* 31621); F, section of apothecium, in water (*Goffinet* 10033). Scales: A, D & E = 2 mm; B = 1 mm; C = 100 μm; F = 200 μm.

taxa from *Tephromela* s. str. through *Calvitimela*, *Violella* and the "*Lecidea*" *aglaea* group to be referred to *Tephromela* s. ampl. but in our opinion this would defeat the purpose of genera to circumscribe like species groups, and make *Tephromela*, which even in its narrow definition has more than 20 described species, unnecessarily large

and unwieldy. We expect "*Lecidea*" *aglaea* will eventually be placed in its own genus, possibly together with *C. perlata* (Haugan & Timdal) R. Sant., which has *Bacidia*-type asci, and some of the various entities currently treated as chemotypes of "*Lecidea*" *aglaea* (Haugan & Timdal 1994). Already Andreev (2004) has postulated that these two taxa are closely related, though he retained them in *Calvitimela*. We leave these problems unresolved until a better sampling of *Calvitimela* s. lat. has been achieved, perhaps including Southern Hemisphere taxa (Fryday 2011) and *Heppsora*, a south Asian genus (Awasthi & Singh 1977), for which DNA could not be obtained for the current study.

Taxonomy

Violella T. Sprib. gen. nov.

MycoBank No: MB 519831

Genus novum ad *Tephromelataceas* pertinet. Generi *Calvitimela* simile sed differt pigmentis hymenialibus violaceis (haud viridibus), ascis ut in *Biatora* constructis (haud ut in *Lecanora*), ascosporis primum hyalinis, demum strato interno fuscescenti (haud persistenter hyalinis) et substanciis chimicis aliis (atranorinum vice acidi alectorialici).

Typus: *Violella fucata* (Stirt.) T. Sprib.

Thallus crustose, areolate to rimose; *photobiont* chlorococcoid algae. Thallus chemistry includes the depside atranorin, a depsidone and a fatty acid.

Apothecia apparently biatorine, macroscopically black, formed on a rudimentary thalline cushion, this prosoplectenchymatous, tawny with brown streaks; *proper exciple* reduced; *epihymenium* not differentiated as a distinct layer, epipsamma lacking; *hymenium* inspersed with violet granules ('Fucatus-violet') that react N+ raspberry red, K+ peacock green; *paraphyses* straight or slightly curved with thinner cross-bridges; *asci Biatora*-type; *ascospores* simple, in the known species two per ascus (reported as occasionally 1 or 3: Stirton 1879; Leighton 1879; James 1971), initially with a single wall, eventually a differentiated internal wall turning brown.

Pycnidia apparently rare, colourless or with light brown pigment around ostiole, sunken in thallus areoles; conidiophores *Parmelia*-type; *conidia* bacilliform.

Etymology. Diminutive of *Viola*, a reference to the characteristic pigment in the hymenium of both known species (Fig. 3).

Comments. Species of *Violella* are distinguished from related genera first and foremost by their abundant Fucatus-violet pigment and the tendency of the inner ascospore walls to become brown. The latter character appears to have been recorded only once previously in the literature, by Leighton (1879: 545), who noted the tendency of the 'protoplasm' of the ascospores of *V. fucata* to turn a 'nigro-fulvaceous colour' in K. However, this colour, apparently produced in the internal ascospore wall, is present even without treatment with K and seems to occur in all mature ascospores in both species of the genus (Figs. 2D, 3C & F, 4A, B & F). It appears that many ascospores with a brown inner wall are collapsing internally and are possibly abortive (Fig. 4A & B). However, healthy ascospores with brown internal walls were also observed (Fig. 4F) and no mature ascospores were observed in either species that had not turned brown internally.

Kantvilas (2009) speculated that the perlatolic acid-containing species of *Mycoblastus*, the so-called *M. dissimulans* group, may constitute a distinct genus. We were not able to sample that group for our phylogeny, but we note that *Violella* differs from *M. dissimulans* in 1) its paraphyses linked over small bridges to other paraphyses, as opposed to the dense network paraphyses similar to those in *Mycoblastus* s. str. formed in *M. dissimulans*; 2) its ascospores, in which the internal ascospore wall frequently becomes brown or olivaceous (remaining hyaline in *M. dissimulans*); and 3) its secondary thallus chemistry. We suspect that *M. dissimulans* will ultimately be found to cluster more closely with *Mycoblastus* s. str., which also has brittle, anastomosing paraphysis networks. We are not aware of any existing generic

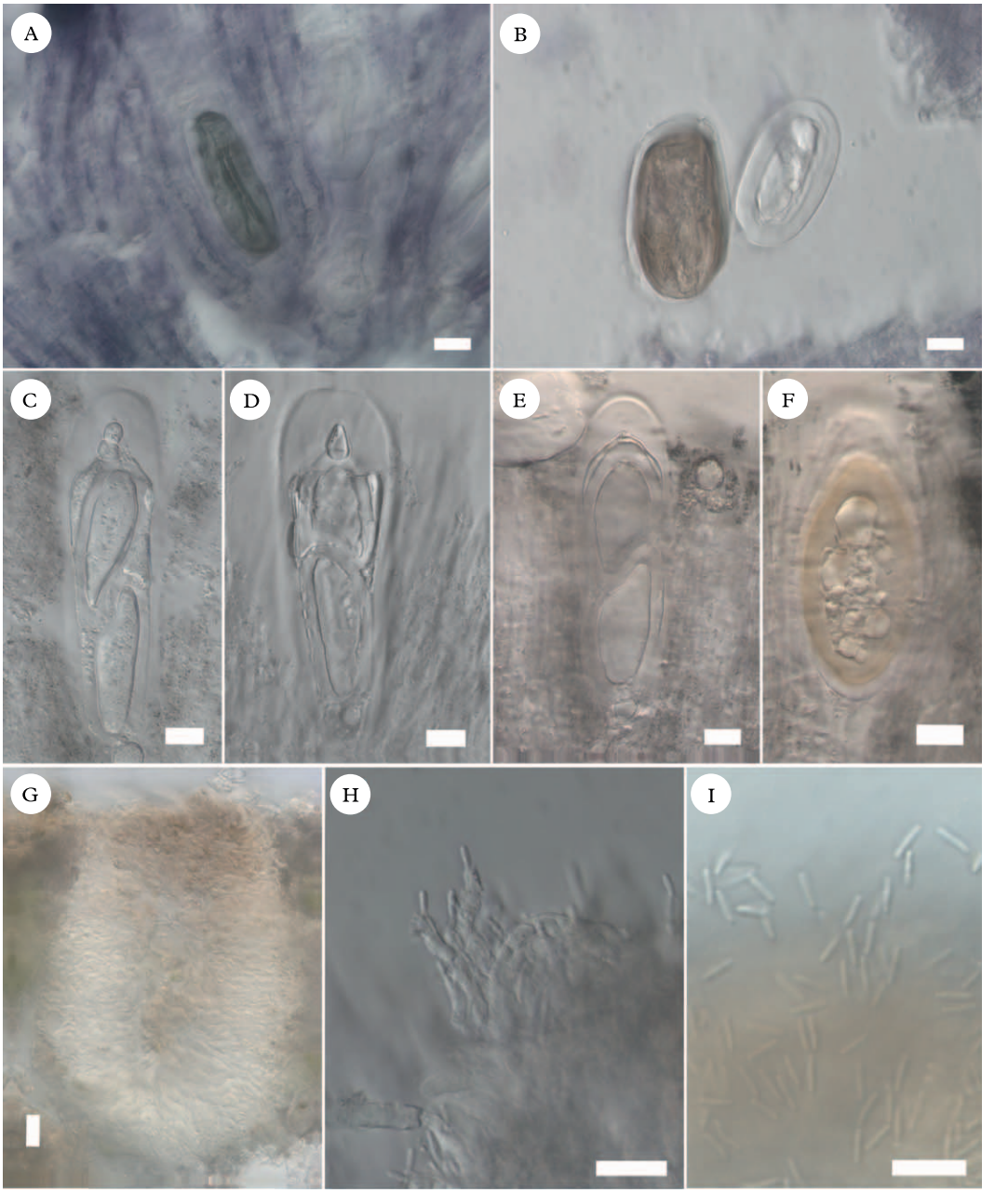

FIG. 4. Microscopic characteristics of *Violella* apothecia and pycnidia. A, *V. fucata* ascospore in water (*Tønsberg* 19004). B–I, *V. wangii* internal anatomy; B, mature and immature ascospore, in water; C–F, asci at different stages of development, ending in nearly mature ascospore, using differential interference contrast following pretreatment with C; G, section of pycnidium in water (holotype); H, conidiophores (in water); I, conidia (in water). B–F from *Goffinet* 10033, G–I from holotype. All scales = 10 μm.

name in this group that would need to be considered before describing *Violella*.

Violella fucata (Stirt.) T. Sprib. comb. nov.

MycoBank No.: MB 519832

Basionym: *Lecidea fucata* Stirton, *Scottish Naturalist* **5:** 16 (1879); type: Great Britain, Scotland, Mid Perth, Tyndrum, on wood, July 1878, *Stirton s.n.* (BM!—holotype).— *Megalospora fucata* (Stirt.) H. Olivier, *Bull. de Géogr. Bot.* **21:** 187 (1911; on p. 207 Olivier incorrectly attributes the combination to Leighton 1879).— *Mycoblastus fucatus* (Stirt.) Zahlbruckner, *Cat. Lich. Univ.* **4:** 3 (1926).

(Figs 3A–C, 4A)

The first species of the genus to be described was *Violella fucata* (Stirton 1879, as *Lecidea fucata*), but this taxon rarely produces apothecia. A detailed description is provided by James (1971). *Violella fucata* is widely reported from western Europe (e.g., Tønsberg 1992; James & Watson 2009), the Pacific Coast of North America (British Columbia and Washington: Tønsberg 1993; Alaska: Spribille *et al.* 2010) and eastern North America (Massachusetts: Spribille *et al.* 2011 and below; Newfoundland: Tønsberg 1993; New York: Schmull *et al.* 2002; Harris 2004). A distribution map of its obligate parasite *Tremella lichenicola* (Diederich 1996: 102) includes many European and some western North American records.

Selected specimens examined. **Norway:** *Hordaland*: Fjell, Sotra, Tælavåg, W of Midttveit, UTM 32V, KM 766874, map 115 IV, alt. 10 m, [corticolous] on maritime *Calluna vulgaris*, 1993, *T. Tønsberg* 19004 (BG, *c.fr.*); Sogn og Fjordane, Askvoll, W of Fure, S of Djupevika, hill 48, UTM 32V, KP 8601, Map 1117 IV, alt. 20–48 m, [corticolous] on *Calluna vulgaris*, 1989, *T. Tønsberg* 11779 (BG, *c.fr.*).—**Great Britain:** *Scotland*: **V.C. 105**, West Ross, Dundonnell, Allt a' Chàirn ravine, on lignum of fallen *Betula* trunk, alt. 160 m, 1999, *A. M. & B. J. Coppins* 18794 (E, *c.fr.*); **V.C. 107**, East Sutherland, N side of Dornoch Firth, Spinningdale, Ledmore Wood, on lignum of fallen, decorticate *Quercus* trunk, alt. 10–30 m, 2001, *B. J. & A. M. Coppins* 20015 (E, *c.fr.*); **V.C. 97**, Westerness, N side Loch Sunart, Coel na mara, on lignum of fallen trunk of *Quercus*, alt. *c.* 40 m, 2004, *B. J. Coppins* 21427 *& H. L. Andersen* (E, *c.fr.*).—**USA:** *Massachusetts*: Berkshire Co., Mount Greylock, 42°38·231′N, 73°10·208′W, 1034 m alt., lignicolous on snags, 2009, *T. Spribille* 32161 *& V. Wagner* (FH, GZU, NY).

Violella wangii T. Sprib. & Goffinet sp. nov.

MycoBank No.: MB 519833

A *Violella fucata* areolis maioribus bullatisque, apotheciis maioribus et substanciis chimicis aliis (atranorinum et acidum roccellicum/angardianum vice atranorini et acidi fumarprotocetrarici) differt. Habitat in montibus altis Asiae extratropicae.

Typus: China, Yunnan, Lijiang Prefecture, Lijiang Co. S of Lijiang, Jinhue village, Laojunshan Mountain, at the border with Jianchuan Co., 26°38·538–37·936′N, 99°43·509–45·992′E, 3510–3900 m, montane forest dominated by *Abies* and further up by *Rhododendron*, along trail from parking lot to peak, epiphytic, 16 July 2010, *B. Goffinet* 10029, with *L. Wang, S. L. Guo and S.Y. Huang* (KUN—holotypus; CONN, GZU—isotypi); same locality, same date, *B. Goffinet* 10033, with *L. Wang, S.L. Guo and S.Y. Huang* (TNS, UPS).

(Figs 3D–F, 4B–I)

Thallus crustose, covering patches as much as 8 cm diam., consisting of discrete areoles (0·15–)0·2–0·6(–1·5) mm diam., these sometimes confluent forming a rimose thallus; colour white to ashen grey, surficial thallus granules corticate, corticate surface finely pruinose; cortex in esorediate thalli prosoplectenchymatous, 30–55 µm thick; algal layer *c.* 50 µm thick, grading into medulla that is variably thin to as much as 200 µm thick, to 300 µm thick under apothecia; *soredia* when present borne in soralia at tips of areoles, rarely areoles dissolving into soredia, internal and external soredia white; soredia roundish, (40–)64–88(–110) µm diam., sometimes forming consoredia; *hypothallus* not observed; *photobiont* chlorococcoid, cells rounded to irregularly angular, (7–)8·4–11·1(–17) µm diam.

Apothecia always present, rounded, single or clustered in groups of 2–3 and becoming confluent, (0·7–)1·3–2·6(–4·1) mm diam., base broadly adnate, disc ± flat to weakly convex, jet black and shiny; margin indistinct, visible from above only in the youngest apothecia, concolorous with the disc; 'thalline cushion' present, rarely visible from above and forming a thin white line, in section prosoplectenchymatous, variable in thickness, 25–230 µm thick, typically tawny brown with streaks of darker brown pigment, clearly differentiated from subhymenium above and

medulla below; *proper exciple* similar in structure to the hymenium, hyphae radiate, similar to paraphyses, when well developed in young apothecia to 170 μm wide laterally, filled with Fucatus-violet granules and often suffused with Cinereorufa-green pigment; differentiated hypothecium absent; *subhymenium* consisting of a thin layer of ascogenous hyphae, *c.* 20–50 μm tall, filled like the hymenium with Fucatus-violet granules but sometimes also infused with Cinereorufa-green; *hymenium* highly variable in thickness even within one and the same apothecium, (80–) 100–300(–350) μm tall, strongly infused with Fucatus-violet granules and collectively forming a deep violet impression in section, but hymenial gel itself hyaline in thin section; *epithecium* not differentiated, epipsamma lacking; *paraphyses* mostly simple, arranged vertically and linked to each other in their lower halves by thin bridges, the main beams stouter than the bridges and not readily breaking when squashed in K; paraphysis tips not or scarcely expanded, 4–6 μm wide including gel sheaths, lumina to 1·5 μm wide, paraphyses completely coated on the outside by Fucatus-violet granules; *asci* clavate, 85–110 × 25–33 μm when mature, inner and outer walls staining blue, tholus strongly I_{Lugol}+ blue, pierced by a broad, conical non-amyloid structure, thus similar to the *Biatora*-type; *ascospores* 2 per ascus, beginning colourless and apparently with a single wall, eventually developing a secondary inner wall, which quickly turns brown while still in the ascus; outer wall thick, to 5 μm in some cases, the inner brown wall thin, often collapsing (spore aborting?), live, healthy ascospores also with brown endospore, (35–)41·7–54·2 (–65) × (15–)20·8–30·8(–35) μm in water.

Pycnidia apparently rare, barely visible externally, in small colourless bumps on the thallus, to 60 μm diam.; wall 10–20 μm thick, pigmented a pale rufous brown or hyaline; *conidiophores* of *Parmelia*-type (type VI of Vobis 1980), with zig-zag cells sprouting conidia in upper part of each cell; *conidia* bacilliform, *c.* 4–5 × 1 μm.

Chemistry. Atranorin and roccellic/angardianic acid detected by TLC.

Etymology. The species is named in honour of Dr. Wang Li-Song, for his ongoing efforts to describe the lichen diversity in western China.

Habitat and distribution. Found on bark of *Rhododendron* sp. in China (Hengduan Shan, Yunnan) and on wood of *Pinus pumila* in the Russian Far East (Bureinskiy Khrebet, north-western Khabarovskiy Krai). Substratum was not recorded for the Indian and Bhutanese material. Collections came from elevations of 3500 to 4000 m in the southern area and *c.* 1000 m in the northernmost collection. In two of the collections it was associated with *Mycoblastus affinis*; one of these specimens is included in our phylogeny.

Comments. *Violella wangii* is a distinct species that seems to be widespread, if rarely collected, in the mountains of high Asia. It occurs in two intergrading morphs, one esorediate with granular, corticate areoles that can become heaped and almost phyllocladioid, and another in which these areoles remain small and erupt in apical soralia, in one specimen even disintegrating completely into soredia in parts of the thallus. The two morphs exhibit no other consistent differences however and several specimens are intermediate. The apparently fluid gradient between esorediate and sorediate morphs recalls the case of *Mycoblastus sanguinarius* (Tønsberg 1992), in which fully leprose morphs have not been found to be genetically distinct from esorediate morphs (T. Spribille, unpublished data).

Violella wangii differs from the only other species in the genus, *V. fucata*, in possessing much larger thalli (frequently covering patches 4–8 cm in diam. (rarely >3 cm diam. in *V. fucata*), robust areoles 0·2–0·6 mm across (to 0·3 mm in *V. fucata*), external soredia, if present, which remain white (often turning bluish grey in *V. fucata*), and chemistry (roccellic/angardianic instead of fumarprotocetraric acid). Ascospores average larger in *V. wangii* than in *V. fucata*; though based on a limited number of apothecia available and paucity of ascospores, our measurements in *V. fucata* (38·5 ± 6·7 ×

18·5 ± 3·3 μm, $n = 24$) fall exactly within the ranges given by Stirton (1879) and James & Watson (2009). The apothecia of *V. wangii* are larger than anything we have measured in *V. fucata* but this may not be a reliable character given that apothecia are rare and often poorly developed in *V. fucata*, a primarily sterile species.

Specimens examined (*V. wangii*). **Bhutan:** *Tongsa District*: Black Mountains NW of Nubji, 27°12′N, 90°22′E, 4040 m elev., *Rhododendron* thicket with *Abies densa* at treeline on ridges, on *Rhododendron*, 2000, *G. & S. Miehe* 00-13-07/06 (GZU).—**India:** *Darjeeling*: Phalut-Dentam, 11 v 1960, *Togashi et al.* s.n. (TNS). *Sikkim*: Jongri, elev. 4000 m, 20 v 1960, *Togashi et al.* s.n. (TNS).—**Russia:** *Khabarovskiy Krai*: Chegdomyn-Sofiysk road, high pass, watershed between Niman and Umal'ta Rivers, *c.* 7·1 km S of the bridge over the Niman River, 26 km (air line) SW of Sofiysk, 52°05·866′N, 133°42·433′E, *Pinus pumila-Rhododendron aureum* woodland under *Larix gmelinii*, on hard wood of *P. pumila*, 1016 m, 2009, *T. Spribille* 31621 *& L. Yakovchenko* (H).

Vězda (1993) issued an exsiccate of a specimen from China under the name *Mycoblastus fucatus*, but as Kantvilas (2009) has pointed out, it is distinct from that taxon. It was collected near the type locality of *V. wangii* but is distinct from that species in its chemistry (fumarprotocetraric instead of roccellic/angardianic acid, in this respect recalling *V. fucata*) and thallus morphology (larger, flatter areoles). It is also distinct from the chemically concordant *V. fucata* in, amongst other characters, developing larger thalli, large, flattened areoles and large apothecia, and apparently lacking soredia. We regard this as probably another species distinct from *V. wangii* and *V. fucata* based on thallus chemistry and morphology. However, we were unable to obtain fresh material of this species and hesitate to describe it without getting a better overview of its variability. We have seen three specimens conforming to this morphology and chemistry, all from China.

Specimens examined (unnamed fumarprotocetraric acid-containing form): **China:** *Prov. Yunnan*: montes Yulong Shan, 30 km ad septentriones ab oppido Likiang, alt. 4000 m s.m., 25 vii 1990, *Soják* s.n. (Vězda, *Lich. Rar. Exs.* 66, GZU). *Prov. Sichuan*: Hengduan Shan, Daxue Shan, 57 km S of Kangding, Gongga Shan, Hailougou glacier and forest park, 29°34′35″N, 101°59′56″E, 2940–3130 m, on *Betula utilis*, 2000, *W. Obermayer* 08686 (GZU*); ibid.*, northern Qionglai Shan, Barkam, 31°57′N, 102°39′E, 4050 m, 1995, *G. & S. Miehe* 94-502-2/14 *& U. Wündisch* (GZU).

Status of *Mycoblastus indicus*

A candidate name for our new taxon that required examination was *Mycoblastus indicus* (Awasthi & Agarwal 1968, as "*indicum*"), described from Darjeeling district, India, near to where *Violella wangii* has also been collected. We did not receive a response to repeated requests for type material from Lucknow (LWU), but we did find a specimen of *M. indicus* at UPS, collected and identified by Awasthi and Agarwal only days before they collected the type specimen. The specimen fits the description provided by Awasthi & Agarwal (1968) and in habit resembles the photograph of the holotype provided by Singh & Sinha (2010), though the latter appears to have more mature apothecia. *Mycoblastus indicus* is clearly not a member of *Mycoblastus* or *Tephromelataceae*. Instead, detailed study of the UPS specimen (Fig. 5) revealed brown epihymenial and hypothecial pigments, a strongly developed proper exciple, mostly simple, loose paraphyses, and asci with a dark apical amyloid cylinder. We obtained an unknown phenolic substance from the thallus, with R_f values similar to confluentic acid in TLC. We concur with Awasthi & Agarwal's original statement that the species appears similar to the group of tropical species around *Lecidea granifera* Vain., for which the genus *Malmidea* has been erected by Kalb *et al.* (2011). We accordingly combine the species into that genus, where it appears similar to *M. coralliformis* Kalb. We note that it has larger ascospores than any of the members of the genus discussed by Kalb *et al.* (2011).

Malmidea indica (Awasthi & Agarwal) Hafellner & T. Sprib. comb. nov.

Basionym: *Mycoblastus indicum* Awasthi & Agarwal, *Current Science* **37:** 84 (1968); type: India, Darjeeling District, Pashkok Road, 19 March 1967, *D. D. Awasthi & M. R. Agarwal* 67.78 (LWU—holotype, *n.v.*).

Specimen examined (*Malmidea indica*). **India:** *Darjeeling District*: Rangit river valley, near Lebong, alt. 1520 m, on bark of trees, 1967, *D. D. Awasthi & M. R. Agarwal* 67.224 (UPS).

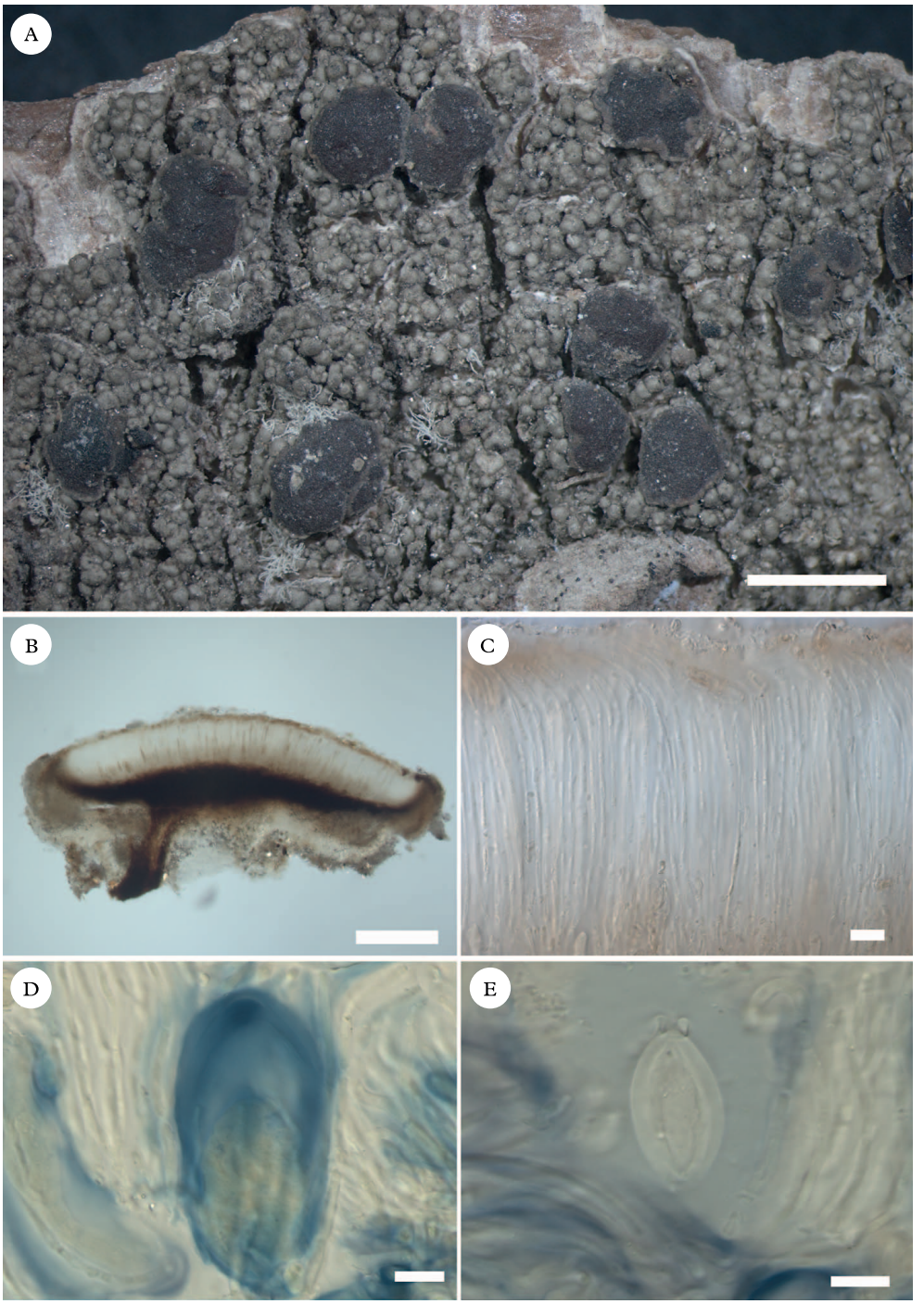

FIG. 5. *Malmidea indica* (*Awasthi & Agarwal* 67.224, UPS). A, habit; B, section of apothecium; C, section through hymenium showing paraphyses; D, ascus, squash preparation stained in $I_{Lugol's}$ after pretreatment with K; E, ascospore, in $I_{Lugol's}$. Scales: A = 2 mm; B = 200 μm; C–E = 10 μm.

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